Misfolding and aggregation of the amyloid β-protein (Aβ) are hallmarks of Alzheimer's disease. Both processes are dependent on the environmental conditions, including the presence of divalent cations, such as Cu^{2+}. Cu^{2+} cations regulate early stages of Aβ aggregation, but the molecular mechanism of Cu^{2+} regulation is unknown. In this study we applied single molecule AFM force spectroscopy to elucidate the role of Cu^{2+} cations on interpeptide interactions. By immobilizing one of two interacting Aβ42 molecules on a mica surface and tethering the counterpart molecule onto the tip, we were able to probe the interpeptide interactions in the presence and absence of Cu^{2+} cations at pH 7.4, 6.8, 6.0, 5.0, and 4.0. The results show that the presence of Cu^{2+} cations change the pattern of Aβ interactions for pH values between pH 7.4 and pH 5.0. Under these conditions, Cu^{2+} cations induce Aβ42 peptide structural changes resulting in N-termini interactions within the dimers. Cu^{2+} cations also stabilize the dimers. No effects of Cu^{2+} cations on Aβ-Aβ interactions were observed at pH4.0, suggesting that peptide protonation changes the peptide-cation interaction. The effect of Cu^{2+} cations on later stages of Aβ aggregation was studied by AFM topographic images. The results demonstrate that substoichiometric Cu^{2+} cations accelerate the formation of fibrils at pH 7.4 and 5.0, whereas no effect of Cu^{2+} cations was observed at pH 4.0. Taken together, the combined AFM force spectroscopy and imaging analyses demonstrate that Cu^{2+} cations promote both the initial and the elongation stages of Aβ aggregation, but protein protonation diminishes the effect of Cu^{2+}.

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Quantitative Nanomechanical Characterization to Osteocytic Osteolysis

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Introduction

Osteocytes are the spider-shape cells, embedded in the bone matrix [1]. They are considered as the final evolution cells from the osteoblast lineage and dominated in all bone cells. As they are buried into the mineralized bone matrix and hard to access, little attention has been paid to them, in comparison of bone surface cells, osteoblasts and osteoclasts. Traditionally, osteocytes are thought to communicate with each other and other bone surface tissue via their dendrites. With the development of osteocyte technology, it is believed that this kind of cell are involved in regulating bone remodeling, such as sensing of mechanical strains and micro-environmental conditions, to regulating osteoblast and osteoclast activities . More importantly, osteocyte has osteocytic osteolysis function to control bone mineralization and phosphate reabsorption, which is essential for skeletal health and numerous cellular mechanism. However not all of facts favor the osteocytic osteolysis proposal. The argument is still there.

In this work, for the first time we obtained quantitative nanomechnical maps of bone matrix around osteocytes, with novel developed dynamic nanomechanical atomic force microscopy [2, 3]. By comparing the Young's modulus of different locations, extra evidence, from the mechanical point of view, to the osteolysis function has been found.

1. Teti, A. and A. Zallone, *Do osteocytes contribute to bone mineral homeostasis? Osteocytic osteolysis revisited.* Bone, 2009. **44**(1): p. 11-16.
2. Dong, M. and O. Sahin, *A nanomechanical interface to rapid single-molecule interactions.* Nat Commun, 2011. **2**: p. 247.
3. Dong, M.D., S. Husale, and O. Sahin, *Determination of protein structural flexibility by microsecond force spectroscopy.* Nature Nanotechnology, 2009. **4**(8): p. 514-517.

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Alignment of Integrin Surface Receptors with Z-Discs in Live Cardiomyocytes Revealed with High Resolution AFM Mapping

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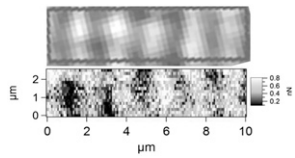
Mechanical sensing proteins connect with sarcomeric structures, such as the Z-disc, which impacts cardiomyocyte mechanotransduction. The extracellular matrix (ECM) plays a vital role acting as a passive scaffold and signaling network.

We used atomic force microscopy (AFM) to map integrin receptor arrangement on adult rat ventricular myocytes (ARVM) to resolve ECM coupling surface receptor alignment with underlying sarcomere structures, particularly the Z-discs. Plated ARVM's were probed by an Asylum MFP-3D AFM with a silicon nitride cantilever (0.1 N/m) and pyramidal tip (radius ~ 40 nm). Tip-surface adhesion forces were measured in scanning mode through a 10 μm X 2.5 μm (78 nm X 156 nm resolution) region. Bare gold and laminin (10 μg/ml) coated tips were used.

Adhesion force maps from bare tip (Fig. 1 bottom) show clear banding patterns that register with features in the bright field image (Fig. 1 top) and have 1.72 μm sarcomere spacing. Peak adhesion forces are generally twice as large for the laminin coating relative to the bare tip, indicative of surface receptor binding. This AFM methodology offers a tool for characterizing a pathway for sarcomere-ECM coupling.



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Local Mechanical Response in Biological Thin Films and Nanostructures

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Mechanical properties can be diagnostic of internal structure in a complex material, but mechanical testing can be challenging for biomaterials, which tend to involve small structures and relatively thin films. We present recent findings on mechanical response in protein aggregates and thin films, demonstrating the benefits of new methods which are non destructive and well-suited to biological systems.

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Variable Cellular Conduct of Photonic Carbon Nano-Dots

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Recently the smaller Quantum dots (Qdots) (< 10 nm) have shown improved cellular imaging over previous generations of Qdots, however the metal content in their formulation still hinders their progress towards translational venue. Carbon dots (Cdots) are novel carbon based nanoparticles which have shown promise as replacements for Qdots. However, their cellular uptake and in vivo imaging applicability are not well-known. Herein, following our work with other multifunctional nanoprobes and nano-delivery systems, we have functionalized cdots and used combined atomic force microscopy (AFM) and fluorescence imaging to show them to be of less than 10 nm in size and equally photo-stable. Moreover, we show their cellular uptake in various cancerous, non-cancerous and stem cell types using confocal and two-photon fluorescence imaging. Quantitative Nanomechanical mapping (QNM) analysis via bio-AFM was carried out to probe the cellular response in presence of Cdots. Cdots have the potential to impact in both health and environmental applications because of their low cost, ready scalability, excellent chemical stability, biocompatibility, colloidal stability, and resilience of PL in vivo.

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The Art of Perceiving Your Sample with AFM-STED-FCS

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The art of learning from a biophysical interest in a scientific problem has emerged as finding the right technical unit capable of searching the interest and tuning the method into a subtle interrogation procedure thereby collecting all the information possible in one single trial. This idea of multi-capable system enamoured our interests in making such a tool capable of asking a few questions to lipid layers and if demanded, ask more in the same test. Potential biophysical interests in elasticity, particle clustering and hi resolution targeting led us into making a hybrid tool capable of AFM.STED.FCS of a custom volume with a high spatial precision.

STED microscopy has been predominant as a super resolution imaging technique and when coupled to an AFM, the new hybrid modality ultimately works with high precision capability which was described last year(1). This precision modality steps into another level by the ability of looking into temporal fluctuations by a spot variable FCS (2) in order to produce a multi questionable nano chamber, inside your sample. This option of multi-questioning can be very useful in addressing elastic measurements, diffusion characterization and sub-diffraction targets. We also drive our measurement interests in membrane studies in nano scale in order to test drive the new hybrid and these results are discussed and presented in this work.

(1)"A novel nanoscopic tool by combining AFM with STED microscopy", B. Harke, J.V. Chacko, H. Haschke, C. Canale and A. Diaspro; Optical Nanoscopy, 2012
(2)"Exploring single-molecule dynamics with fluorescence Nanoscopy",C. Ringemann, B. Harke, C. von Middendorff, R. Medda, A. Honigmann, R. Wagner, M. Leutenegger, A. Schoenle, S.W. Hell, C. Eggeling, New Journal of Physics, 2009